

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
19 September 2002 (19.09.2002)

PCT

(10) International Publication Number
WO 02/072423 A1

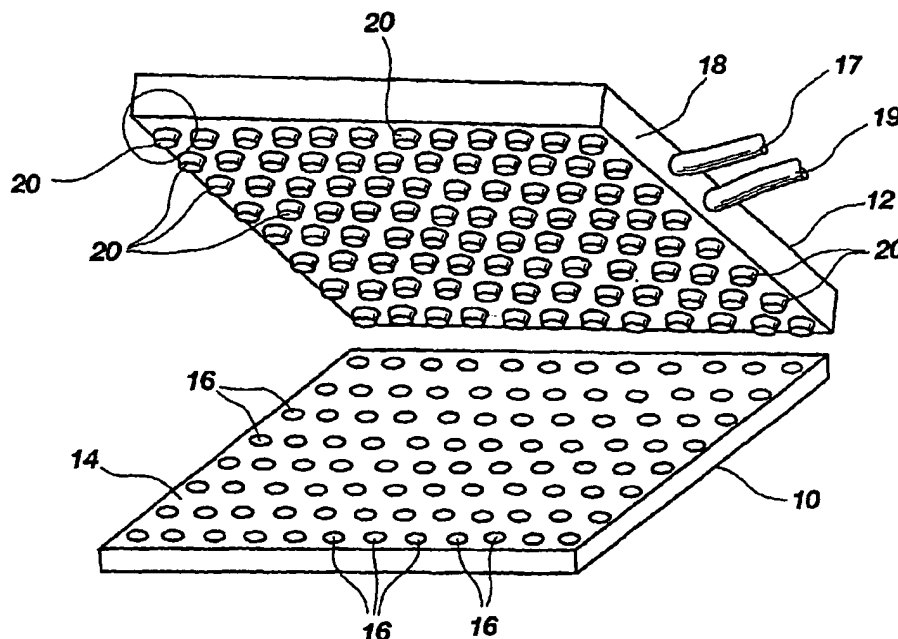
- (51) International Patent Classification⁷: **B65B 1/04** (74) Agent: WITT, Evan; Madson & Metcalf, 15 West South Temple, Suite 900, Salt Lake City, UT 84101 (US).
- (21) International Application Number: PCT/US02/06942
- (22) International Filing Date: 7 March 2002 (07.03.2002)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
60/274,390 9 March 2001 (09.03.2001) US
- (71) Applicant (for all designated States except US): **BIOMICRO SYSTEMS, INC.** [US/US]; 1290 West 2320 South, Suite D, Salt Lake City, UT 84119 (US).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): **MCNEELY, Michael** [US/US]; 2159 East Powderkeg Drive, Sandy, UT 84093 (US). **ADEY, Nils** [US/US]; 2218 Emerson Avenue, Salt Lake City, UT 84108 (US).
- (81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.
- (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declaration under Rule 4.17:

— of inventorship (Rule 4.17(iv)) for US only

[Continued on next page]

(54) Title: MICROPLATE LID



(57) Abstract: A microplate lid (12) containing fluid processing and transport structures is disclosed that provides controlled delivery of small volumes of samples and reagents to a microplate (10), and prevents evaporation during processing.

WO 02/072423 A1

WO 02/072423 A1



Published:

— with international search report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

MICROPLATE LID

BACKGROUND OF THE INVENTION

Field of the Invention

5 The present invention relates generally to the field of laboratory equipment, and particularly to multi-well microplates used to contain multiple fluid samples for chemical and biological reactions or processing steps. More specifically, the present invention relates to a novel lid for use with such microplates, which includes microfluidic circuitry to facilitate delivery of fluid to microplate wells, and which functions in combination with the
10 microplate to provide additional or enhanced fluid processing.

Description of Related Art

Microplates are common laboratory hardware used for numerous liquid based chemical and biological reactions. The standard microplate, or microtiter plate, contains 96 wells in an 8x12 row and column pattern, with the wells having 9 mm center-to-center
15 spacing. Microplates are also available with 384 wells and 1536 wells. The overall dimensions of the plates do not vary very much from type to type. The well-to-well spacing may vary, but is always a multiple of the 9 mm of the standard microtiter plate, such as 4.5 mm for the 384 well plate, and 2.25 mm for the 1536 well plate. A typical 96 well microplate is indicated by reference number 10 in FIG. 1. The volume and shape of the
20 wells within a given plate are usually uniform, but there are many different types of microplates that may have considerably different well shapes and volumes, depending on their intended use. Microplates are made of various materials (typically rigid plastics) depending on their intended applications, and may have biological or chemical coatings suitable for the chemistries to be performed in them.

25 In addition to the commonly used microplates described above, it is also possible to obtain strips of wells that conform to the typical 9 mm well spacing of the microtiter plate, but that include only one column of (for example) 8 wells attached together, or two 8-well columns for a total of 16 wells, or sometimes one or more columns of 12 wells. These strips can also often fit in the various instruments designed for handling and processing
30 microplates, which include heaters, optical plate readers, plate washers, and pipetting equipment, either manual or robotic.

Fluid is typically added to or removed from the wells of microplates using manual or automated pipettors. Lids are often placed on microplates to enclose the fluid within and minimize loss of fluid due to evaporation. Such lids are relatively simple structures, formed

of rigid or flexible polymeric materials that fit over the microplate. Some form a tight seal with the microplate; some do not. It is also common to cover microplates with adhesive film to prevent evaporation or spilling of well contents.

As noted above, a common laboratory task is to load liquid samples into the wells of a microtiter plate. This may be done by manually pipetting the samples into each well, or by using robotic pipetting systems to perform the task. Manual and robotic pipetting devices typically have multiple pipette tips spaced at intervals compatible with standard well spacings. Such devices greatly facilitate the loading of samples and reagents into microplates. However, they are not particularly effective when it is necessary to load very small fluid volumes into microplate wells. It is usually extremely difficult to manually load very small volumes, such as 0.5 μ l, into the microplate wells. It is also difficult for a robotic pipettor to load such small volumes repeatably and reliably onto a plate. Moreover, even if such small volumes can be loaded successfully, evaporation may easily alter the composition of the drop. Even though some robotic systems are capable of delivering volumes down to about 1 μ l reliably, to reduce evaporation the liquid sample may be covered with oil, and when oil is present a larger liquid sample volume may be needed in order to reliably pipette samples out of the well. Thus the practical lower limit for sample volume may be undesirably large in some instances. It would thus be desirable to reduce the practical minimum liquid volume that could be reliably transferred in and out a microplate wells.

Because microplates are well-established and commonly used laboratory devices that are compatible with various types of other laboratory equipment, it would be desirable to provided enhanced function to microplates, such as improved sample and reagent fluid processing capability, while retaining the same basic format for compatibility with existing equipment.

SUMMARY OF THE INVENTION

This invention relates to the construction and use of a microfluidic interface microplate lid in which microfluidic structures are incorporated into a microplate lid to provide for the delivery of liquid to and removal of liquid from the wells of the microplate in finely regulated quantities. Lids constructed according to the invention may also provide the functions of metering, mixing or dividing sample aliquots, perform complex distribution of fluid, allow for pressurization of reactions that take place in the wells of the microplate or within the lid itself, and numerous other functions. The body of the inventive lid is thick enough to include various fluid processing, control, or sensing structures. The lid further

includes multiple projections on its underside, which fit into the microplate wells to seal the wells and provide controlled transport of fluid and gas to and from the wells.

The fluidic processing that takes place within the lid may be controlled by the use of passive fluid control technology or remote valving, as described in previously filed,
5 commonly-owned U.S. Patent 6,296,020, issued October 2, 2001, and PCT International Patent Publication No. WO 02/12734 (which are incorporated herein by reference), or active control mechanisms, such as mechanical valves, electrokinetics, or any combination of these methods. Active sensing, e.g., of fluid location or temperature, may also take place. If the plate is to be heated, the well contents may be pressurized, through the lid, to
10 reduce evaporation and prevent boiling. The general approach of controlling pressure in a reaction chamber has been described previously in Applicant's PCT International Patent Publication No. WO 01/88204, which is incorporated herein by reference. This and the two previously mentioned commonly owned patents are incorporated herein by reference, in their entirety.

15 The invention includes microfluidic interface microplate lids that fit either entire 96 well, 384 well, or 1536 well plates, single column or multiple column strips that comprise a portion of a microplate, or any other multiple or shape of fluid containment device used in liquid analysis.

The microfluidic interface microplate lid can take a fluid volume injected into it and
20 aliquot it into the number of desired wells. Multiple samples can be injected, as desired, and the division of the samples need not be equal. One μ l or less may be delivered, and the fluid will not evaporate because the well is covered and its exposure to air is controlled. There is no need for oil to be deposited to reduce evaporation.

If complex tasks of mixing, dilution, incubation, or chemical reactions are necessary,
25 they may be done in the microplate lid, or within the wells of the microplate. The wells of a microplate or strips may be pre-loaded with reagents and possibly dried, and then liquid would be delivered to the wells for a reaction to take place. Products of a reaction performed in one well may be extracted and delivered to another well for further processing. The lid may be designed to extract liquid from a well and deliver it to an integrated
30 microdetection system, such as a microelectrophoresis system, or, by removing the lid, the fluid in the well may be available for conventional extraction and transfer to another external system. Micro beads often used in some chemical processes could also be present and easily contained in a well.

If fluidic processing requires thermal cycling, or incubation, the plate and lid may be placed in a microplate heater. The fixture holding the plate may be specialized to fit the lid, and may contain pumping and control mechanisms to run the fluidic processing.

5 It is an object of the invention to provide for the delivery of small, finely controlled volumes of fluid to be delivered to the wells of microplate. This is accomplished by using microfluidic processing circuitry within the microplate lid to divide sample into aliquots and deliver aliquots to the wells. This has the benefit of reducing the amounts of sample and reagents used.

10 Another object of the invention to allow the removal of small, finely controlled volumes of fluid from microplate wells. This is achieved by the inclusion of fluid-removal microchannels that projects into wells to remove fluid from the wells.

Another object of the invention is to regulate the venting of air from microplate wells. This is achieved by providing air vents that communicate with microplate wells in the microplate lid. It is further possible to apply a back pressure to the air vent in order to regulate pressure in the well to prevent boiling and/or reduce evaporation

15 A further objective of the invention is to modify the volume of wells in a microplate by providing a lid with protrusions that extend into and partially fill the wells, thereby reducing the available reaction volume. This has the advantage of reducing the air space into which evaporation of sample may occur.

20 Yet another object of the invention is to provide for the mixing of sample or reagent fluids with other fluids or solids prior or subsequent to delivery to microplate wells. This is accomplished through the use of microfluidic processing circuitry in the microplate lid. Mixing of fluids can be used advantageously to dilute samples or reagents or combine materials for various pre- or post-processing reactions.

25 A related object of the invention is to provide for incubation and reaction steps to be carried out in the microplate lid before or after delivery to microplate wells. This objective is accomplished by providing reaction wells within the microplate lid, and further by providing a mechanism for controlling reaction conditions within the wells to carry out the desired reaction. This has the advantage of making it possible to perform multiple reaction steps without moving fluid samples from the microplate to another device.

30 Yet another object of the invention is to provide a microplate lid which includes active elements such as mechanical valves, sensors, heating elements, and the like, in order to perform complex fluid handling and processing steps in a conventional microplate format.

These and other objects of the invention are described more fully in the detailed description herein below.

BRIEF DESCRIPTION OF THE DRAWINGS

In order that the manner in which the above-recited and other advantages and objects of the invention are obtained will be readily understood, a more particular description of the invention briefly described above will be rendered by reference to specific embodiments thereof, which are illustrated in the appended drawings. Understanding that these drawings depict only typical embodiments of the invention and are not therefore to be considered to be limiting of its scope, the invention will be described and explained with additional specificity and detail through the use of the accompanying drawings in which:

FIG. 1 illustrates a typical 96-well microtiter plate and a microplate lid constructed according to the present invention;

FIG. 2 is a close-up view of a single well-interface protrusion of a microplate lid constructed according to the present invention;

FIG. 3 is a cross section of a three-layer well-interface protrusion positioned in a microplate well;

FIG. 4 is a cross section of a two-layer well-interface protrusion positioned in a microplate well;

FIG. 5 is a perspective view of an interface layer of a microfluidic microplate lid showing channels leading into and out of a well-interface protrusion;

FIG. 6 is a perspective view of an protrusion of a microplate lid having channels for delivering fluid to and extracting fluid from a microplate well;

FIG. 7 is a cross-sectional view of the protrusion shown in FIG. 6;

FIG. 8 is an alternative embodiment of a protrusion for delivering fluid to and extracting fluid from a microplate well;

FIG. 9 illustrates a microfluidic interface microplate lid covering two columns of a standard 96 well microplate;

FIG. 10 illustrates fluid traveling through a first well in a microplate prior to being divided and delivered to two other wells of the microplate;

FIG. 11 illustrates fluid traveling through a well in the microplate lid prior to delivery to two wells of a microplate;

FIG. 12 is a schematic representation of microfluidic circuitry for distributing fluid to sixteen wells of a microplate;

FIG. 13 illustrates a microfluidic interface microplate lid which divides a single fluid stream and provides aliquots to sixteen wells of a microplate; and

FIG. 14 illustrates an alternative microfluidic interface microplate lid that divides a single fluid stream to provide aliquots to sixteen wells of a microplate.

5

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

FIG. 1 illustrates a standard 96-well microplate 10 and a microplate lid 12 constructed according to the present invention. Microplate 10 is, for example, an injection molded plastic structure having a substantially planar upper surface 14 with a plurality of wells 16 disposed therein in a grid pattern. Microplate lid 12 has a substantially planar base structure 18 with a plurality of well-interface protrusions 20 extending from a lower surface thereof. Each well-interface protrusion includes a fluid inlet 17 and an outlet 19, which may provide for either air or liquid to exit the system. Fluid inlet 18 may be connected to an external source of sample or reagent fluids. Outlet 19 may be connected to equipment that will perform further processing of sample fluids or it may provide for the venting of air, or the pressurization of the system. A single inlet 17 and outlet 19 are depicted, but certain embodiments of the inventive microplate lid may include larger numbers of either structure. Well-interface protrusions 20 are configured to fit into wells 16. In the example depicted in FIG. 1, microplate lid 12 covers microplate 10 in its entirety, and a well-interface protrusion 20 is provided to correspond to each of the 96 wells in microplate 10. However, in some embodiments of the invention, there may not be a well-interface protrusion corresponding to each well 16. Individual wells may be skipped, or entire regions of the microplate may be omitted; moreover, it is not necessary that the microplate lid cover the entire microplate. FIG. 2 is a detail view of a single well-interface protrusion 20. Opening 22 and opening 24 in underside 26 of protrusion 20 connect to microchannel 28 and microchannel 30, respectively. Microchannel 28 and microchannel 30 (illustrated by dashed lines) may connect to additional microfluidic circuitry (not shown) in base structure 18 of microplate lid 12. The size and shape of protrusion 20 may vary depending on the application for which the lid is designed.

FIGS. 3 and 4 show cross sections of two embodiments of the microfluidic interface microplate lid, illustrating how it may interface with a microplate. In these figures, a single well-interface protrusion and corresponding microplate well are illustrated. In the embodiment of FIG. 3, microplate lid 12 is formed of three layers. Microplate interface layer 32 contacts the microplate 10 directly and forms the outer surface of well-interface

protrusion 20 that fits into well 16 of the plate. Middle layer 34 lies adjacent microplate interface layer 32 opposite the side that contacts microplate 10. Channels and fluid control elements used in the specific application of the lid are formed at the interface between the microplate interface layer and the middle layer. In FIG. 3, channel 28 with opening 22 and channel 30 with opening 24 are shown. Channel 28 may be an inlet channel that is in fluid communication with a fluid inlet in the microplate lid, and which delivers fluid into space 25 in well 16. Channel 30 may be an outlet channel through which fluid (or air) leave space 25 and is delivered to downstream features, which may include air escape vents, waste fluid reservoirs, fluid outlets, additional microfluidic circuitry, and possibly, other microplate wells. Also shown are channel narrowings 46 and 48, which may function as passive valves. In the embodiment of FIG. 3, microplate interface layer 32 and middle layer 34 are designed to be disposable. A top plate 36, which is designed to be re-usable, fits on top of these two layers. Top plate 36 may contain active elements, such as heating element 37, as shown here, or mechanical valves or sensors, to assist in controlling the lid function. Top plate 36 includes protrusion 50, which fits inside protrusion 52 of middle layer 34, which similarly fits inside protrusion 20 defined by microplate interface layer 32. Protrusion 20 fits closely against the interior of well 16 at sealing region 23, to form a sealed (or substantially sealed) enclosed space 25. Enclosed space 25 may have an air-tight seal or a water-tight seal, depending on the requirements for the device. In the embodiment of FIG. 3, sealing region 23 is a circular region where the edge of interface protrusion 20 contacts the interior of well 16. The seal could be formed along a fairly narrow region 23, as depicted in FIGS. 3 and 4, or the seal could be formed along a larger contact area if the interface protrusion 20 conforms more closely to the interior of well 16.

In the embodiment of FIG. 4, there are only two layers: microplate interface layer 32 and top plate 56, with no middle layer. In this case, channels 28 and 30 and fluid control elements 62 and 64 are formed at the interface between microplate interface layer 32 and top plate 56. It should be noted that in FIGS. 3 and 4, the cross section is taken through a channel between microplate interface layer 32 and the adjacent layer, and therefore these layers are separated by the width of the channel except in the region between opening 22 and opening 24; in others regions of the device, the microplate interface layer directly contacts and is adhered or sealed to the adjacent layer.

The structure of microfluid channels at the interface between the microplate interface layer 32 and the adjacent layer is more clearly illustrated in FIG. 5. FIG. 5 depicts microplate interface layer 32 with upper surface 66 exposed; the adjacent layer is not

shown. Microchannels 68, 70, 72, 28a, 28b and 30 are formed in upper surface 66. Main channel 68 branches at branch point 69 into channels 70 and 72. Main channel 68 may be connected to a fluid inlet, through which fluid is introduced into the base structure of the microplate lid. In the present example, the fluid inlet would connect at the edge of the microplate lid, but the invention may also include one or more fluid inlets that connect to the microfluidic circuitry of the microplate lid from the top plate or via other portions of the microplate lid. Channel 70 connects to channel 28a, which extends into interface protrusion 20a. Channel 72 connects to channel 28b, which extends into interface protrusion 20b. Channel 30 also extends into interface protrusion 20a. Channel 30 may be, for example, a second channel through which fluid exits the well to additional downstream features, or to a downstream fluid outlet, or it may be an air vent that allows air, but not fluid, to escape as the well is filled with fluid. Openings (not shown) at the ends of channels 28a and 30a permit the channels to communicate with the well in which the interface protrusion 20a is positioned. Similarly, opening 22b at the lower surface of interface protrusion 20b provides fluid communication between channel 28b and the well in which protrusion 20b is positioned. When the adjacent layer (which could be either middle layer 34 or top plate 56) is assembled to microplate interface layer 32, the lower surface of the adjacent layer forms the upper surface of the channel structures formed in microplate interface layer 32. Although in the present example the microfluidic channels are formed in the microplate interface layer, and sealed by the adjacent layer, microfluidic structures can be formed in other layers of the microplate lid, as well. Moreover, microfluidic structure may be formed in several, overlapping layers in order to form three-dimensional microfluidic circuit structures.

The protrusions of the microfluidic interface microplate lid are designed to be inserted into the wells of the microplate and to control the volume of the well that is to be used. The protrusion will typically fit closely in and seal with the well. Some protrusions are designed to deliver fluid into a well; others are designed not only to deliver fluid, but also to remove fluid once a reaction has taken place. The lid is designed to control the movement of fluid and air within the well-lid system. Each protrusion has a fluid outlet, through which fluid is delivered to the microplate well, and an inlet, which may be used for the removal of fluid or release of air from the microplate well. By using a smaller diameter inlet, flow of fluid back into the microplate lid from the well may be prevented, while air flow is still permitted. The microfluidic interface microplate lid may include multiple air or

fluid ducts for each well, possibly of different protruding depths and diameters, to allow for whatever fluid control or transport is required.

To facilitate easy filling and extraction of a sample from a well, the protrusion may be in a shape resembling a corkscrew, as shown in perspective view in FIG. 6. In this design, the protrusion 80 is configured to conform closely to the interior surface of the whole well. A spiral groove 82 in the outer surface of protrusion 80 forms a spiral channel, which has an outer wall formed by the wall of the microplate well. Fluid enters the spiral channel from channel 81 that runs between microplate interface layer 88 and middle layer 90, via opening 83. A seal is formed between neck region 85 of protrusion 80 and the interior of the well in which it is placed, as well as along the spiral ridge formed by the exterior surface 87 of protrusion 80. Fluid may be passed through the spiral channel around the exterior of protrusion 80 until it reaches the bottom of the well. Protrusion 80 occupies most of the volume of the well, and the substantially sealed space that will be formed by the spiral channel and the bottom of the well will be relatively small.

As shown in the cross-section of FIG. 7, an opening 84 in the center tip of protrusion 80 connects to a channel 86 that runs between the microplate interface layer 88 and middle layer 90. Channel 86 may be used for removal of fluid from the well. However, the invention is not limited to this particular spiral channel design, and various other channel designs may be used as well. FIG. 8 is a cross-sectional view of a "straw" type design in which fluid may be removed from the well via a straw type extension 94 of protrusion 20. Fluid enters space 95 formed in well 16 via a hole 22, which communicates with channel 28 in interface layer 32. When it is desired to remove the fluid, additional fluid is injected into well 16 through the upper hole 22, thus forcing the fluid already present in space 95 out, through hole 96 in straw type extension 94 and then through channel 30.

As noted previously, it is not necessary for the microfluidic interface microplate lid to interface with all of the wells of a microplate. A microplate lid may be designed to cover and work with only a portion of the wells. FIG. 9 shows an example of a microplate lid 98 that interfaces with only two centrally located rows 100 and 102 of a plate 10. Fluid inlet 101 and outlet 103 are also depicted. Microchannels connecting inlet 101 and outlet 103 with wells 16 are also illustrated.

Alternatively, the microplate lid may cover a region of a plate but not include protrusions that fit into the wells in that portion of the plate. Thus the microplate lid simply passes over certain wells and does not interface with them. This may be done if a well is not needed at that location for a particular application, and/or if the area within the lid is

instead used for some kind of fluid control function. For example, the microfluidic interface microplate lid may contain wells or reaction chambers in addition to those present in the microplate, so that reactions may be performed within the lid. FIG. 10 and 11 show exemplary configurations of microplate wells and microfluidic circuitry (located in the microplate lid overlying the wells). In both examples, microplate wells are represented by large circles, microfluidic channels which carry fluids are represented by heavy lines, and channels for air flow are represented by lighter lines. In the example given in FIG. 10, four wells 104, 106, 108, and 110 are available, but the microfluidic circuitry accesses only three of the wells. Fluid is delivered into well 106 via channel 112, and then it is moved out of well 106 via channel 114, which branches into channels 116 and 118, which deliver fluid to wells 108 and 110, respectively. FIG. 10 gives an example of simple microfluidic circuitry (i.e., for dividing a fluid stream) downstream of microplate well 106, and leading to additional microplate wells (108 and 110). Air ducts 120 and 122, which merge into air duct 123, allow air to escape from wells 108 and 110 as they are filled with fluid. In the example of FIG. 11, two of the wells, 124 and 126, are bypassed. The microfluidic circuitry in the microplate lid includes channel 132 leading to well or reaction chamber 134 for processing of fluids prior to delivery into microplate wells 128 and 130, via channels 136, 138 and 140. Reaction chamber 134 is a simple example of microfluidic processing circuitry that can be located upstream of microplate wells (in this case, wells 128 and 130). As in the circuit of FIG. 10, air is vented from wells 128 and 130 as they are filled via air ducts 142 and 144, which merge into air duct 143. Reaction chamber 134 could contain a reactant or reagent (e.g., in lyophilized form) for performing a pre-processing step with fluid before it is delivered to wells in the microplate.

The most critical part of this system is the point of contact between the microplate well 16 and interface protrusion 20 from interface layer 32. It is important that there is not leakage of fluid between these surfaces. In some applications, leakage of air is to be avoided, as well. This point of contact between the microplate well and protrusions on the interface layer can be reversibly "sealed" by making the interface layer, at least at this location, out of a soft, rubberized, plastic such as silicone rubber or polyurethane. A soft coating on a harder base plastic may also serve this function. It is also useful for this interface to be hydrophobic, so if there is a gap its effect will be minimized. The top plate can be designed so that it provides downward pressure to affect the reversible seal.

The microplate interface layer and middle layer may be sealed together physically, or they may be pressed together by the top plate as described above. It is critical that these

two layers effectively contain fluid within the channels and chambers as designed, and that no leaking takes place. Similar materials and techniques may be used to prevent leakage as described in the previous paragraph.

The middle layer and microplate interface layer may also be designed to “snap” together using a series of interdigitated members that fit together and define the channel space between them. If the microplate were custom made it is also possible that the interface layer and microplate could “snap” together as well. However, it is anticipated that the microfluidic interface microplate lid will most frequently be used with standard, “off the shelf” microplates, and is to be usable without requiring modifications to the microplate design.

FIG. 12 is a schematic of the distribution of a fluid sample to sixteen wells 16 in a 2-dimensional planar substrate. In this illustration, fluid is delivered to the wells via the channels indicated by the darker lines. Fluid enters at inlet channel 142, splitting into first generation daughter channels 144, second generation daughter channels 146, third generation daughter channels 148, and fourth generation daughter channels 150, which lead to individual wells 16. The channels indicated by lighter lines allow air to be displaced from the wells as fluid enters, and, in this case, are not designed to transport fluid. Again, multiple generations of channels 152, 154, 156, and 158 join, eventually all merging to form main air escape channel 160. Passive valving of the type described in U.S. Patent 6,296,020, issued October 2, 2001, or remote valving as described in PCT International Patent Publication No. WO 02/12734, may be used to control the flow of the fluid in channels leading to the wells so the sample may be distributed evenly or aliquoted to the wells in any desired volumes, and to ensure that the sample fills each well without flowing beyond the well.

FIG. 13 and 14 illustrate two possible channel layouts that could be used for distributing fluid in a microfluidic interface microplate lid. The circuit of FIG. 13 is not truly equivalent to the layout of FIG. 12, but is substantially functionally equivalent in the cases where the volume of the sample to be distributed is greater than the total fluid containing volume of the channels and wells combined. In this layout, it can be seen that because inlet channel 170 divides into two channels 172 and 174, each of which delivers fluid to multiple wells 16 in series, it is not possible to deliver fluid to individual wells selectively. Delivery channels 175 extend from channels 172 and 174 to individual wells 16 and branch off of channels 172 and 174 at intervals. If the sample volume is greater than

the total volume of the channels and wells, all wells can be filled uniformly with sample and this relatively simpler layout is sufficient.

However, if the volume of the sample is smaller than the total volume of the channels and the well, a more complicate channel layout that permits more controlled
5 distribution of fluid, as shown in FIG. 14, must be used. This channel layout is equivalent to the schematic layout presented in FIG. 7. A binary branching structure is used so that each well 16 is an equal distance from the inlet channel 170. In order to distribute sample uniformly between the wells, a volume of sample just sufficient to fill all the wells (but not the channels) can be injected into inlet 170 and then followed by a buffer solution or by air
10 to push the sample into the wells and take up the dead volume of the channels in the lid. Valves can be used to direct the flow of fluid at each generation of branches to ensure that fluid is distributed uniformly among the channels and, ultimately, among all the wells 16. In the example of FIG. 14, fluid flows through first generation daughter channels 180 and 182, second generation daughter channels 183, 184, 185 and 186, third generation daughter
15 channels 187, 188, 189, 190, 191, 192, 193, and 194, and fourth generation daughter channels 195, 196, 210 before entering wells 16. However, it can be seen that a strictly binary branching pattern is not used in the air escape ducts leading to main air escape channel 212. Passive valves are used in the presently preferred embodiment of the invention, but remote valves, as discussed above, or other types of valves may be used
20 instead.

In genomic applications, a common task is to fill the wells of a microplate with sample and PCR components and place it in a thermal cycler oven to amplify the DNA. Microfluidic circuitry present in the microfluidic interface microplate lid could be used to distribute the sample and PCR components to each well, seal the fluid within the wells to
25 prevent fluid movement or evaporation, and then push the fluid into additional wells for further processing if desired once PCR is complete. Previous work has described how a plate can be sealed by pressurizing the fluidic system to prevent or eliminate evaporation. As an alternative, the lid could be mechanized so that it physically shuts off the well outlet channels. In order to perform these reactions in a thermal cycler machine, it might be
30 necessary to modify the machine slightly to accept the microplate with the lid. Other DNA amplification techniques exist that are isothermal. This would be similar to an incubation step, which can also be handled with the microfluidic interface microplate lid. The microfluidic interface microplate lid may include active heating or cooling elements, or both, to reduce thermal gradients that may reduce the effectiveness of the thermal process

taking place in the microplate wells. Various methods could be used to heat or cool the lid, as are known by those familiar with this technology.

The microfluidic interface microplate lid may contain various active elements such as plungers to open or close channels or to push fluid, and it may contain other types of mechanical valving, pumps, heaters or sensors.

If the microplate interface layer is made of a hydrophobic material, then when the lid is removed from the plate the tendency of the fluid would be to remain in the microplate well and not to splash around or cling to the interface layer. This is very advantageous when access to the fluid in the wells is desired.

The top plate of the microfluidic interface microplate lid would generally be made of durable materials, such as metal or hard plastics, may have several permanent fixtures, such as valves and sensors, and may be a machined or injection molded piece. The microplate interface layer and middle layer may be formed by embossing, blow molding, injection molding, or other similar processes. Some of the features in these layers could be laser machined as well. The materials for these layers must be selected to provide physical and chemical properties suitable for the application for which they are designed. Such parameters would be known to someone skilled in the art of materials manufacturing.

The present invention may be embodied in other specific forms without departing from its structures, methods, or other essential characteristics as broadly described herein and claimed hereinafter. The described embodiments are to be considered in all respects only as illustrative, and not restrictive. The scope of the invention is, therefore, indicated by the appended claims, rather than by the foregoing description. All changes that come within the meaning and range of equivalency of the claims are to be embraced within their scope.

CLAIMS:

1. A microplate lid comprising:
 - a. a substantially planar base structure;
 - b. at least one fluid inlet to provide for the introduction of fluid into said base structure;
 - c. at least one well-interface protrusion projecting from a lower surface of said base structure, said protrusion adapted to fit into a well of a microplate and form an enclosed space between said protrusion and said well; and
 - d. at least one channel passing through said protrusion and said base structure providing fluid communication between said enclosed space and said at least one fluid inlet.
2. The microplate lid of claim 1, wherein said protrusion is capable of forming an air-tight seal with said well, so that said enclosed space is an air-tight space.
3. The microplate lid of claim 1, wherein said protrusion is capable of forming a water-tight seal with said well, so that said enclosed space is a water-tight space.
4. The microplate lid of claim 1, wherein said enclosed space has a defined volume smaller than the volume of said well.
5. The microplate lid of claim 1, further comprising at least a second channel passing through said protrusion and said base structure providing communication between said enclosed space and a downstream feature in said base structure.
6. The microplate lid of claim 5, wherein said downstream feature comprises a connection to a mechanism for regulating the pressure within said enclosed space.
7. The microplate lid of claim 6, wherein said downstream feature comprises a valve which may be used to block the escape of air through said second channel.
8. The microplate lid of claim 6, wherein said downstream feature comprises a pressure source for applying a backpressure to said second channel.
9. The microplate lid of claim 5, wherein said second channel comprises an air escape duct, and wherein said downstream feature comprises an air escape vent.
10. The microplate lid of claim 5, wherein said second channel comprises a fluid channel, and wherein said downstream feature comprises a waste fluid reservoir.
11. The microplate lid of claim 5, wherein said second channel comprises a fluid channel, and wherein said downstream feature comprises a fluid outlet.

12. The microplate lid of claim 5, wherein said second channel comprises a fluid channel, and wherein said downstream feature comprises a channel delivering fluid to another microplate well via another protrusion.
13. The microplate lid of claim 5, wherein said base structure further comprises valves which can be set to close said first channel and said second channel.
14. The microplate lid of claim 1, wherein said base structure further comprises at least one microfluidic circuit component connected between said at least one fluid inlet and said enclosed space.
15. The microplate lid of claim 14, wherein said at least one microfluidic circuit component is a channel, a well, or a valve.
16. The microplate lid of claim 15, wherein said at least one microfluidic circuit components is a well containing a reagent or reactant.
17. The microplate lid of claim 14, wherein said microfluidic circuit component is adapted to divide fluid entering said at least one fluid inlet into two streams.
18. The microplate lid of claim 14, wherein said microfluidic circuit component is adapted to mix two fluid components.
19. The microplate lid of claim 18, wherein said microfluidic circuit component performs the function of diluting a sample prior to delivery to a microplate well.
20. The microplate lid of claim 1, wherein said base structure further comprises at least one active element.
21. The microplate lid of claim 20, wherein said at least one active element comprises a heating element, a cooling element, an electrode, a valve, or a sensing device.
22. A microplate lid comprising:
- a. a substantially planar base structure;
 - b. at least one fluid inlet to provide for the introduction of fluid into said base structure;
 - c. a plurality of well-interface protrusions projecting from a lower surface of said base structure, each said protrusion adapted to fit into a well of a microplate and form an enclosed space between said protrusion and said well; and
 - d. a plurality of channels branching from said at least one fluid inlet in a branching pattern within said base structure, said branching pattern in fluid communication with a plurality of microplate wells via at least a portion of said plurality of well-interface protrusions.

23. The microplate lid of claim 22, wherein said branching pattern comprises:
- a. a branch point at which a channel in fluid communication with said at least one fluid inlet splits into at least a first channel and a second channel; and
 - b. a plurality of delivery channels branching off said first channel at intervals and in series, each said delivery channel extending from said first channel through a single protrusion to deliver fluid to the enclosed space formed between said protrusion and its associated well.
24. The microplate lid of claim 23, wherein said first channel is configured to deliver fluid to microplate wells in a single row or column of said microplate.
25. The microplate lid of claim 22, wherein said branching pattern comprises:
- a. at least one channel in fluid communication with said at least one inlet;
 - b. said plurality of channels connected to said at least one channel in a binary branching pattern having at least one generation, wherein at each generation at least one current generation daughter channel splits into two subsequent generation daughter channels.
26. The microplate lid of claim 22, wherein said base structure further comprises at least one active element.
27. The microplate lid of claim 26, wherein said at least one active element comprises a heating element, a cooling element, an electrode, a valve, or a sensing device.
28. The microplate lid of claim 22, wherein said branching pattern causes sample loaded into said fluid inlet to be divided into metered portions and delivered to said plurality of microplate wells.
29. The microplate lid of claim 22, wherein said branching pattern causes sample loaded into said fluid inlet to be divided uniformly among said plurality of microplate wells.
30. The microplate lid of claim 22, wherein said branching pattern comprises one or more valves adapted to direct the flow of fluid through said channels.
31. The microplate lid of claim 30, wherein said valves are selected from passive valves, remote valves and mechanical valves.
32. A microplate lid comprising:
- a. a microplate interface layer comprising a plurality of interface protrusions extending outward from a first side of said microplate interface layer, each said interface protrusion adapted to fit into a well of a microplate and form an enclosed space between said protrusion and said well;

- b. a middle layer adjacent said microplate interface layer on the side opposite said first side of said microplate interface layer;
 - c. a top plate adjacent said middle layer on the side opposite said microplate interface layer;
 - 5 d. at least one fluid inlet formed in at least one of said microplate interface layer, said middle layer, and said top layer;
 - e. at least one microfluidic structure formed in at least one of the interface between said microplate interface layer and said middle layer and the interface between said middle layer and said top layer and in fluid
 - 10 communication with said fluid inlet; and
 - f. a channel providing fluid communication between said at least one microfluidic structure and said enclosed space.
33. The microplate lid of claim 32, comprising microfluidic structures formed in both the interface between microplate interface layer and said middle layer and the
- 15 interface between said middle layer and said top layer.
34. The microplate lid of claim 32, wherein said enclosed space comprises a spiral channel defined by a spiral groove formed in the outer surface of said interface protrusion.
35. The microplate lid of claim 32, further comprising a second channel providing fluid
- 20 communication between said enclosed space and a downstream feature in said microplate lid.
36. The microplate lid of claim 35, wherein said second channel comprises a straw-type extension of said interface protrusion.
37. The microplate lid of claim 32, wherein said microplate interface layer is formed of
- 25 a soft, rubberized plastic.
38. The microplate lid of claim 32, wherein said microplate interface layer is coated on its bottom surface with a soft, rubberized plastic.
39. The microplate lid of claim 32, wherein said top plate comprises at least one active element.
- 30 40. The microplate lid of claim 39, wherein said at least one active element comprises a heating element, a cooling element, an electrode, a valve, or a sensing device.
41. The microplate lid of claim 32, wherein said middle layer and said interface layer snap together.

42. The microplate lid of claim 32, wherein said microplate interface layer is formed of a hydrophobic material.
43. The microplate lid of claim 32, wherein at least a portion of said microplate interface layer is coated with a hydrophobic material.
- 5 44. The microplate lid of claim 32, wherein said at least one microfluidic structure comprises a hydrophobic passive valve.
45. The microplate lid of claim 32, wherein said top layer is formed by machining or injection molding, and wherein said microplate interface layer is formed by embossing, blow molding, injection molding, or laser machining.
- 10 46. A microplate lid comprising:
- a. a microplate interface layer comprising a plurality of interface protrusions extending outward from a first side of said microplate interface layer, each said interface protrusion adapted to fit into a well of a microplate and form an enclosed space between said protrusion and said well;
 - 15 b. a top plate adjacent said microplate interface layer on the side opposite said first side of said microplate interface layer;
 - c. at least one fluid inlet formed in at least one of said microplate interface layer, and said top layer;
 - d. at least one microfluidic structure formed at the interface between said
20 microplate interface layer and said top plate and in fluid communication with said fluid inlet; and
 - e. a channel providing fluid communication between said at least one microfluidic structure and said enclosed space.
47. The microplate lid of claim 46, wherein said enclosed space comprises a spiral
25 channel defined by a spiral groove formed in the outer surface of said interface protrusion.
48. The microplate lid of claim 46, further comprising a second channel providing fluid communication between said enclosed space and a downstream feature in said microplate lid.
- 30 49. The microplate lid of claim 48, wherein said second channel comprises a straw-type extension of said interface protrusion.
50. The microplate lid of claim 46, wherein said microplate interface layer is formed of a soft, rubberized plastic.

51. The microplate lid of claim 46, wherein said microplate interface layer is coated on its bottom surface with a soft, rubberized plastic.
52. The microplate lid of claim 46, wherein said top plate comprises at least one active element.
- 5 53. The microplate lid of claim 46, wherein said at least one active element comprises a heating element, a cooling element, an electrode, a valve, or a sensing device.
54. The microplate lid of claim 46, wherein said microplate interface layer is formed of a hydrophobic material.
- 10 55. The microplate lid of claim 46, wherein at least a portion of said microplate interface layer is coated with a hydrophobic material.
56. The microplate lid of claim 46, wherein said at least one microfluidic structure comprises a hydrophobic passive valve.
57. The microplate lid of claim 46, wherein said top layer is formed by machining or injection molding, and wherein said microplate interface layer is formed by
15 embossing, blow molding, injection molding, or laser machining.

1/6

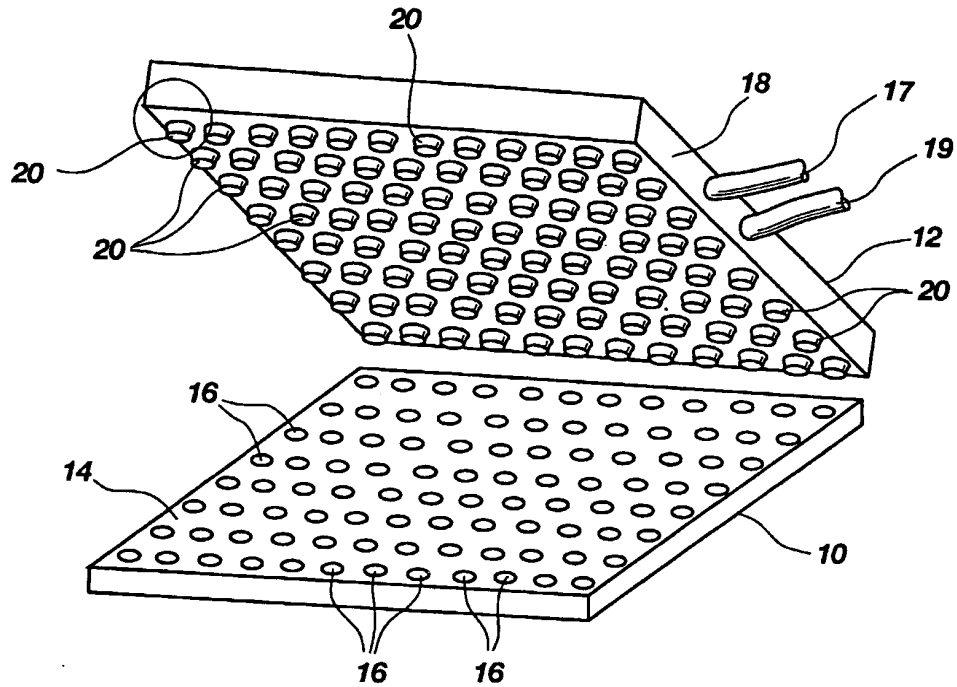


Fig. 1

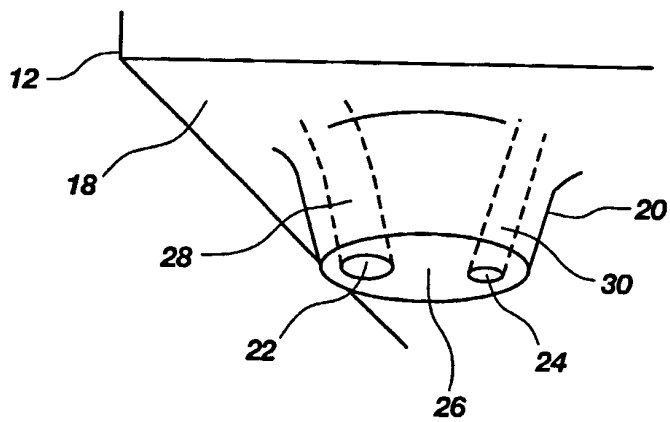


Fig. 2

2/6

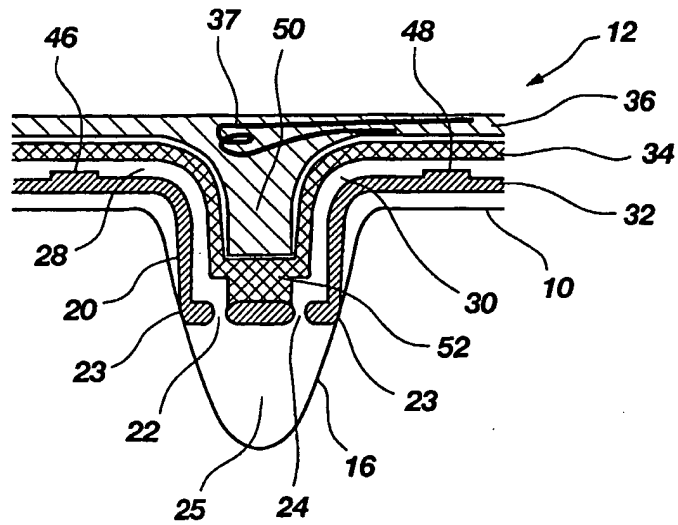


Fig. 3

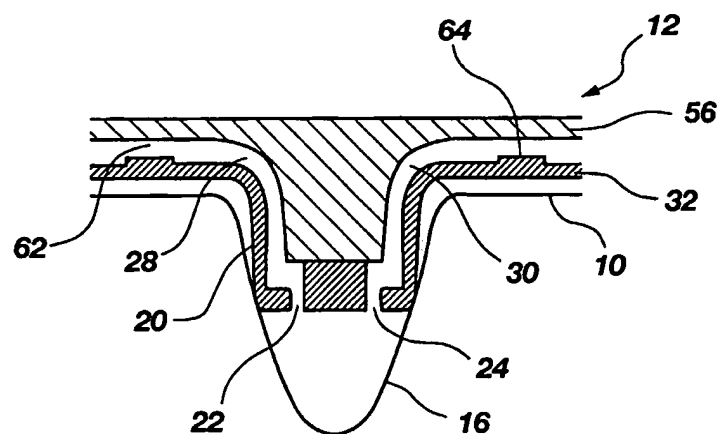


Fig. 4

3/6

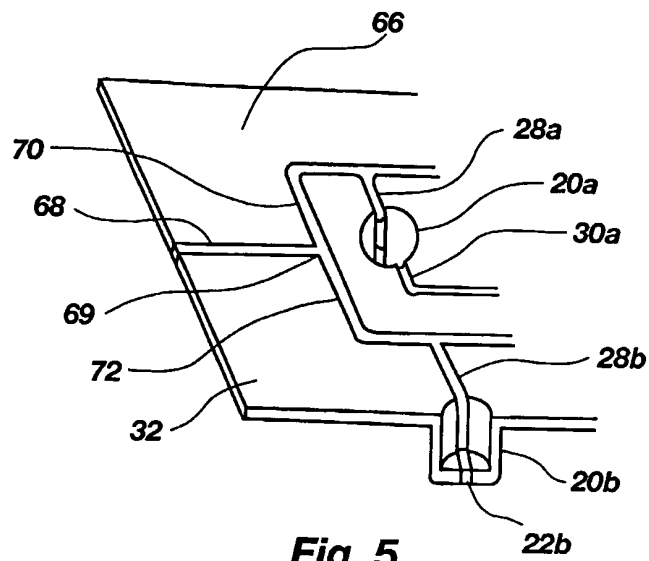


Fig. 5

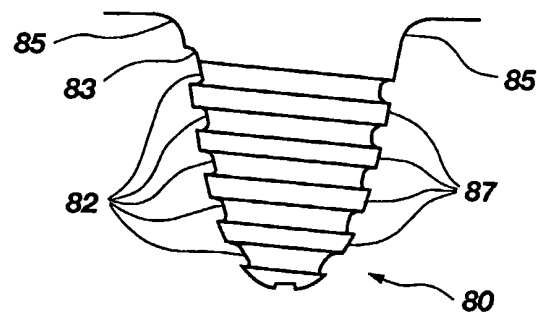


Fig. 6

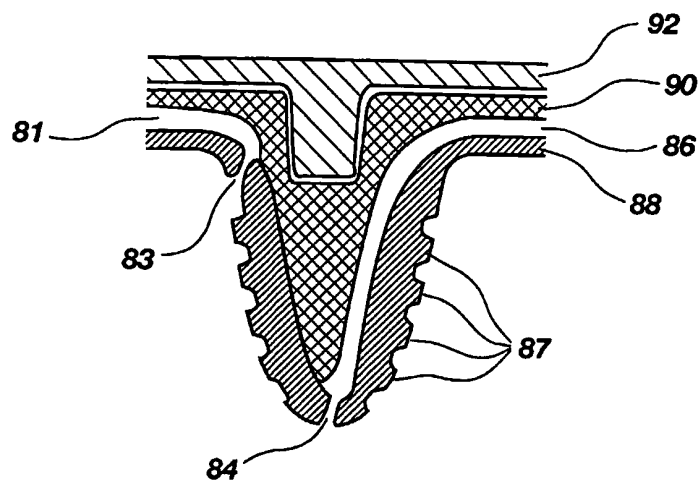


Fig. 7

4/6

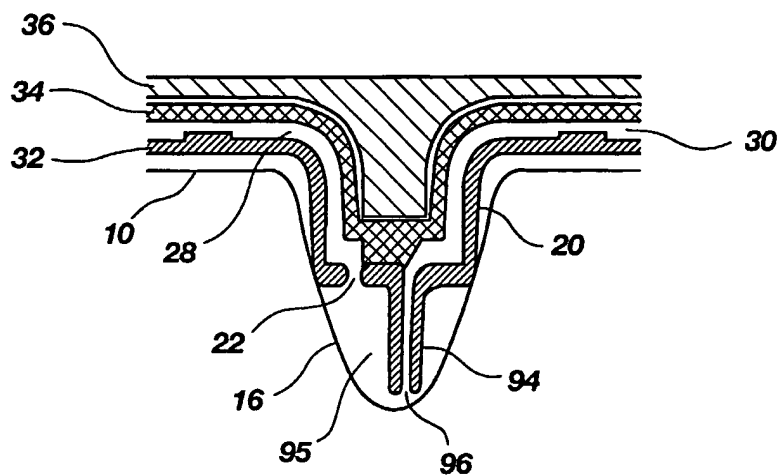


Fig. 8

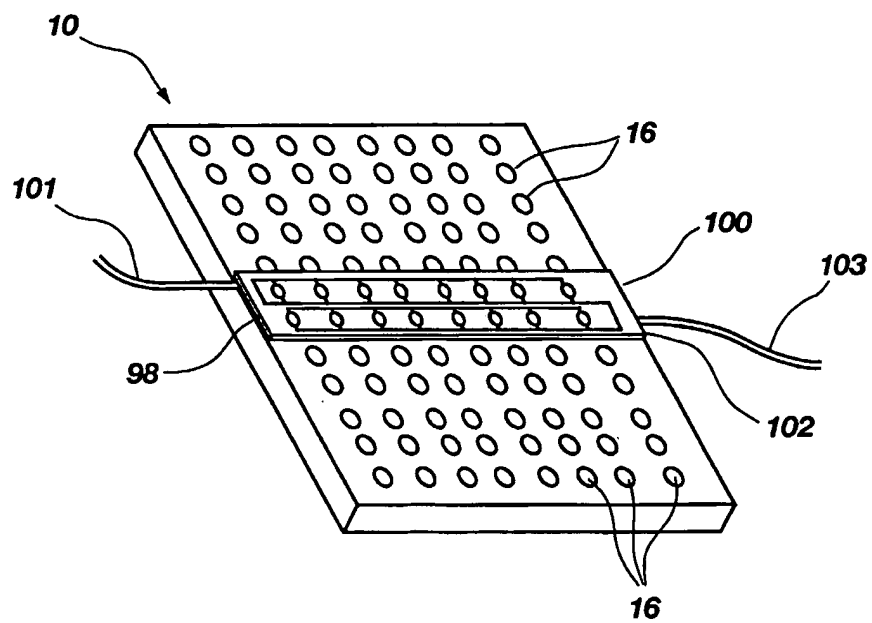
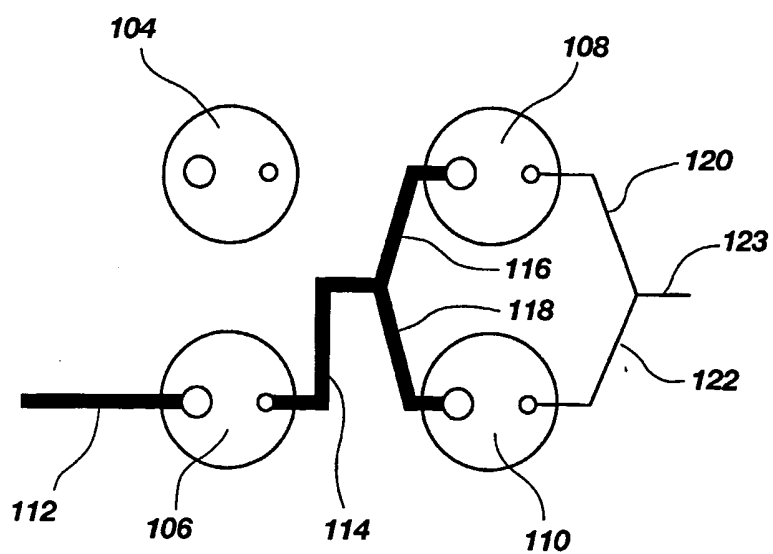
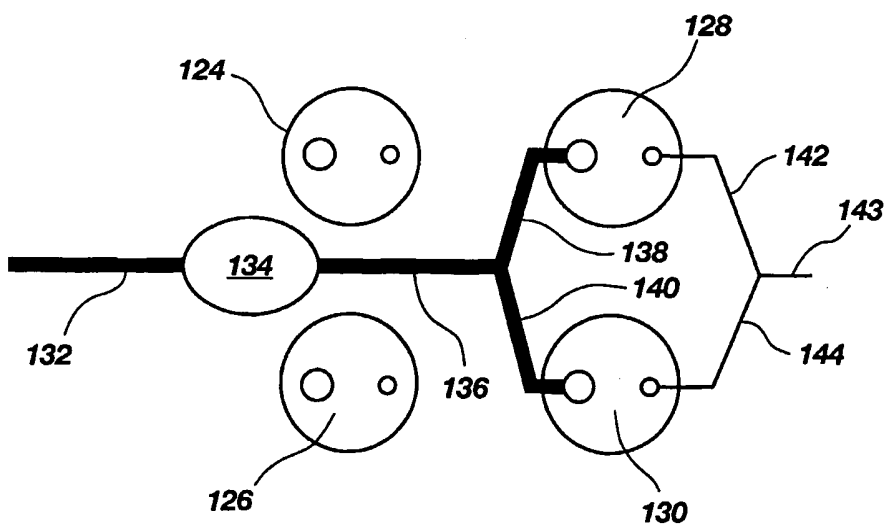


Fig. 9

5/6

**Fig. 10****Fig. 11**

6/6

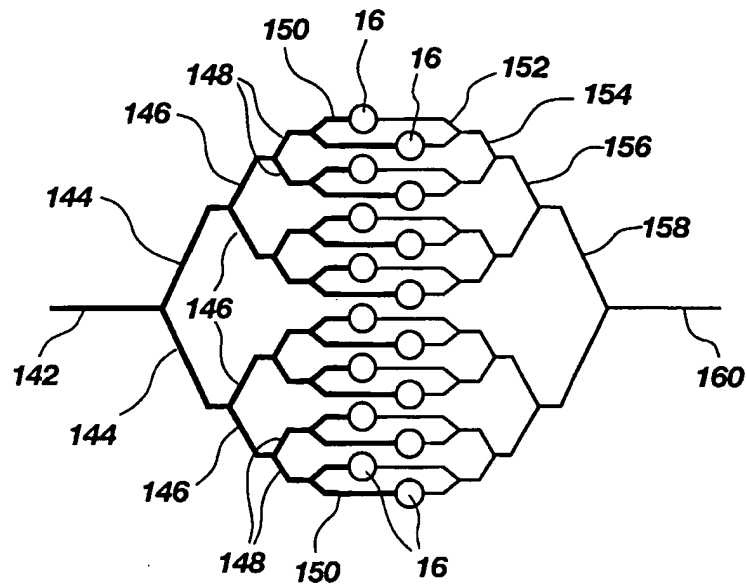


Fig. 12

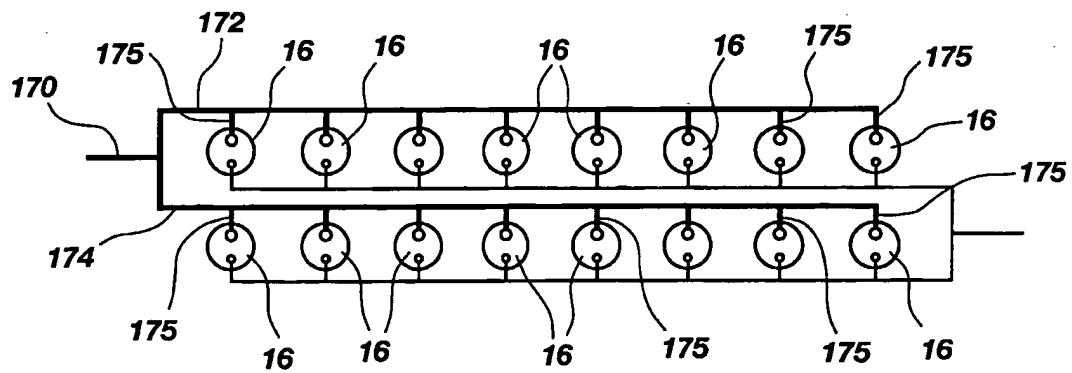


Fig. 13

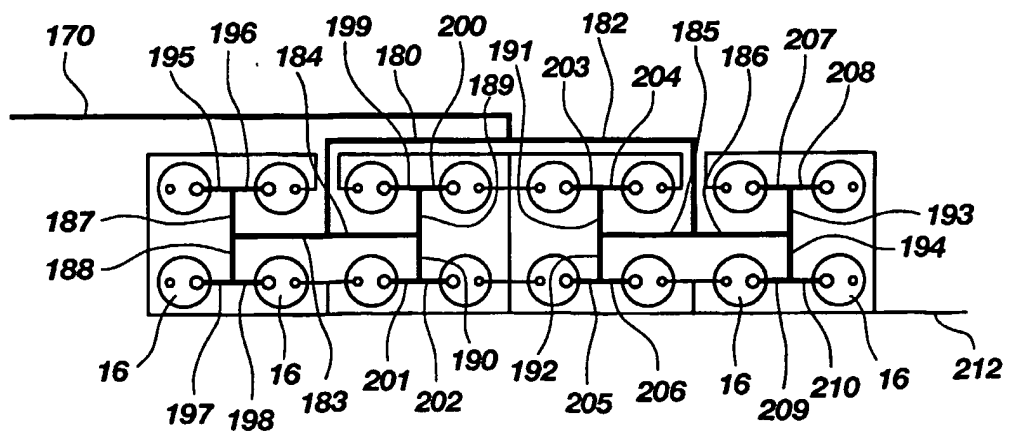


Fig. 14

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/06942

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : B65B 1/04

US CL : 141/129; 435/305.3

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 141/129; 435/305.3, 305.2; 422/99-102

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
none

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EAST

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|---|-----------------------|
| A | US 5,817,510 A (PANDEY et al.) 06 October 1998 (06.10.1998), see entire document. | 1-57 |
| A | US 4,304,865 A (O'BRIEN et al.) 08 December 1981 (08.12.1981), see entire document. | 1-57 |
| A | US 5,459,300 A (KASMAN) 17 October 1995 (17.10.1995), see entire document. | 1-57 |

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

| Special categories of cited documents: | |
|---|--|
| "A" document defining the general state of the art which is not considered to be of particular relevance | "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention |
| "E" earlier application or patent published on or after the international filing date | "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone |
| "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| "O" document referring to an oral disclosure, use, exhibition or other means | "&" document member of the same patent family |
| "P" document published prior to the international filing date but later than the priority date claimed | |

Date of the actual completion of the international search

02 May 2002 (02.05.2002)

Date of mailing of the international search report

31 MAY 2002

Name and mailing address of the ISA/US

Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. (703)305-3230

Authorized officer

Steven O. Douglas

Telephone No. 703-308-0861